PATENT

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Applicant(s):	Matthew During *et al.*
Application No:	09/863,179
Filing Date:	May 23, 2001
Entitled:	Glutamic Acid Decarboxylase (GAD) Based Delivery Systems
Atty. Docket No:	102182-12
Confirmation No:	9640

Group Art Unit: 1632

Examiner: A.M. Falk

Certificate of Mailing (37 C.F.R. 1.8(a))

I hereby certify that this correspondence is being deposited with the United States Postal Service Post Office as first class mail in an envelope addressed to: Commissioner for Patents, Washington, D.C. 20231 on the date set forth below.

Nov. 12, 2003		By:
Date of Signature and Mail Deposit		George A. Xixis Reg. No: 38,664

Commissioner for Patents
P.O. Box 1450
Alexandria, VA 22313-1450

RULE 132 DECLARATION OF DR. MICHAEL KAPLITT

I, Michael G. Kaplitt, residing at 515 East 72nd Street, Apt. 34D, New York, New York, hereby declare as follows:

1. I received a Bachelor of Arts degree from Princeton University in Molecular Biology in 1987, a Tri-Institutional MD-PhD degree from the The Rockefeller University and Cornell University Medical College in 1993 and 1995. During my postdoctoral training at the Rockefeller University in the Laboratory of Biochemical Genetics and Metabolism, I was also an

intern in surgery and a resident in neurosurgery from 1995-1999. I was a Chief Resident in Neurosurgery at the New York Hospital–Cornell University Medical College from 1999-2000. I was also a Clinical Fellow in the Department of Stereotactic and Functional Neurosurgery at the University of Toronto from 2000-2001. I was appointed Director of the Laboratory of Molecular Neurosurgery, The New York Hospital-Cornell University Medical College and Fellow, The Rockefeller University from 1995-2000. I am currently Assistant Professor of Neurosurgery and Director, Laboratory of Molecular Neurosurgery, Weill Medical College of Cornell University, NY, NY; a Clinical Assistant Attending, Division of Neurosurgery, Dept. of Surgery, Memorial-Sloan Kettering Cancer Center, NY, NY; and an Adjunct Assistant Professor, Laboratory of Neurobiology and Behavior, The Rockefeller University, NY, NY. A copy of my Curriculum Vitae more fully explaining my qualifications, publications and appointments is attached as Exhibit A. I am an inventor on the above-referenced patent application.

2. I am familiar with the patent application at issue and, through this declaration, I present the evidence that was requested and discussed during the interview with Examiner Falk, at the United States Patent and Trademark Office, on October 24, 2003. In particular, evidence relating to the broader application of the invention to different neurological disorders or diseases.

3. The claimed invention relates to methods for altering expression of a glutamic acid decarboxylase (GAD) in a region of the brain . This is accomplished by identifying a target site in the central nervous system that requires modification and delivering a vector that comprises a nucleic acid sequence encoding glutamic acid decarboxylase (GAD) to target site of the central nervous system (e.g., a region of the brain), to alter expression of GAD in the region of the brain.

4. The invention also demonstrates the principal that expression of GAD in a region of the brain alleviates the symptoms of Parkinson's disease rodent and primate *in vivo* models for Parkinson's Disease.

5. One of the embodiments of the invention demonstrates that GAD transduction of neurons in the subthalmic nucleus (STN) increases inhibition in the substantia nigra (SN) and decreases the excitatory effect of STN stimulation on neurons in the SN. The results show that changing the excitatory projection from the STN to the SN into an inhibitory projection, using a

gene therapy approach, alleviates the symptoms of Parkinson's disease (*See* page 59, lines 16-20).

6. The same result was repeated in our extended study, the results of which are published by Luo *et al.* in Science "Subthalmic GAD Gene Therapy in a Parkinson's Disease Rat Model" (2003) 298: 425-429 (Exhibit B). This paper demonstrates that GAD gene transfer into glutamatergic excitatory neurons leads to an inhibitory bias with altered network activity. This phenotypic shift provides strong neuroprotection and demonstrates there is plasticity between excitatory and inhibitory neurotransmission in the mammalian brain that results in a therapeutic effect.

7. The same inventive concept of delivering GAD to a region of the central nervous system, can be applied to any CNS disease in which increasing GABA production is desirable.

8. As further evidence that the invention can be applied to different diseases -- as well as different regions of the CNS -- a post-graduate student working at Neurologix, Inc., (licensee of the present invention) carried out the same method disclosed in the instant application in an animal model of epilepsy. This work, described below, shows that the symptoms of epilepsy can be reduced by delivering GAD to a region of the brain involved in epilepsy, e.g., the hippocampus.

9. The epilepsy experiment involved delivering three AAV vectors, AAV/CBA-hGAD65-1.76-WPRE-BGH ("GAD65"); AAV/CBA-hGAD67-WPRE-BGH ("GAD65"); and AAV-EGFP ("EGFP"), into three experimental groups of animals. A fourth sham group ("SHAM") of animals, was subject to injection with an empty needle as a control for injury related to insertion of a needle into the brain tissue. Two microliters of each of the AAV vector, at a genomic titer of 2 x 10^{10} genomes/ml, was infused bilaterally into the rat hippocampus using stereotaxic surgery.

10. Four weeks after vector administration, 10mg/kg kainic acid (i.p.) was administered to each animal to induce seizures. The animals were observed for the next 90 minutes for a variety of behavioural characteristics and by electroencephalograms of seizure activity. Figure 1 (Exhibit C) shows the results from an electroencephalogram of seizure activity

in the hippocampus of a rat kainic acid model for epilepsy. The data shows that rats treated with GAD, in particular, GAD65, have reduced seizures compared with the rats treated with EGFP or the SHAM group that received no vector. The results show that expression of GAD in a region of the brain associated with epilepsy provides neuroprotection against seizures.

11. Further evidence that GAD can be delivered to a selected region of the CNS is presented in Boulis *et al.*, "Stereotactic Gene Based Hypothalamic Neuromodulation" (2002) AANS meeting, Chicago (abstract) (Exhibit D).

12. In Boulis *et al.* an AAV-GAD construct, disclosed in the instant application, was used to deliver GAD to the lateral nucleus of the hypothalamus of rats to augment GABA production in the region. The delivery of GAD resulted in altered gene expression and sustained enhancement of GABA production in a deep brain target, which resulted in an alteration of metabolic behavior.

13. Another example of how GAD can be delivered to selected regions of the brain is shown in Jasmin *et al.* in Nature, "Analgesia and hyperalgesia from GABA-mediated modulation of the cerebral cortex" (2003) 424:316-320. (Exhibit E).

14. In Jasmin *et al.*, GABA neurotransmission in the rostral agranular insular cortex (RAIC) of freely moving rats, was altered by locally increasing GABA using two methods: (a) an enzyme inhibitor; and (b) a double-cassette-defective Herpes Simplex Virus (HSV) vector. Use of gene transfer mediated by a viral vector produced lasting analgesia in the rats by enhancing the descending inhibition of spinal nociceptive neurons. This reference further evidences that a variety of vectors may be used to deliver GAD in a targeted manner and alter GABA levels in relevant regions of the CNS.

15. A further example showing that delivering GAD to a region of the central nervous system, may be applied to any relevant disease is evidenced by a recent article by Levanthal *et al.* in Science "GABA and Its Agonists Improve Visual Cortical Function in Senescent Monkeys" (2003) 300:812-815 (Exhibit F).

16. Leventhal *et al.* demonstrated that the alteration of GABA levels, in a region of the visual cortex (V_1) of aged primates resulted in improved acuity -- including improved orientation and direction selectivity, decreased spontaneous activity and an increased ability to signal visual stimuli. This is further evidence that methods of altering GABA levels, such as delivering GAD to the central nervous system, can be used to address a variety of neurodegenerative diseases.

17. Thus one of ordinary skill in the art, would be able to use the application's disclosure, in addition to the knowledge available in the art, to apply the invention to alter expression of glutamic acid decarboxylase (GAD) in a selected region of the brain.

18. In summary, the disclosure in the application, in combination with the knowledge available in the art, would enable one skilled in the art to perform the full scope of the claimed invention without undue experimentation.

19. I further declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 10001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the application or any patent issuing thereon.

Dated: 11/6/07

Michael G. Kaplitt, M.D., Ph.D.

1268185.2